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Simulating the formation of biofilms in an undergraduate modeling course

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Abstract

Meaningful applications that illustrate fundamental concepts and techniques are crucial in computational science education. In this paper, we discuss development of a simulation on the structural growth of a biofilm that is appropriate for modeling, simulation, or high performance computing courses. Consideration of cellular automaton simulations, boundary conditions, and diffusion in this context can empower students to develop similar simulations for other applications. Moreover, extensions of the basic model can illustrate and motivate the need for high performance computing in computational science. The module, "Biofilms: United They Stand, Divided They Colonize," used for instruction and developed by the authors as an Undergraduate Petascale Education Program (UPEP) Curriculum Module is available at http://computationalscience.org/upep/curriculum.

Keywords: computational science; education; modeling; simulation; high performance computing; biofilms

1. Introduction

Wofford College [1] offers an introductory course, Modeling and Simulation for the Sciences (COSC 201), whose only prerequisite is Calculus I. Besides these two courses, Bachelor of Science students obtaining the Emphasis in Computational Science (ECS) take Introduction to Programming and Problem Solving (in Python), Data Structures (in Python and C++), and Data and Visualization and do a summer internship involving computation in the sciences [2]. With *Introduction to Computational Science: Modeling and Simulation for the Sciences* [3] as a text, half of the Modeling and Simulation course covers system dynamics modeling using a tool, such as *STELLA*®, *Vensim*®, or *Berkeley Madonna*®. The second half of the semester employs a computational tool, such as *Mathematica*®, *Maple*®, or *MATLAB*®, for developing cellular automata (CA) simulations, where the world under consideration consists of a rectangular grid of cells, and each cell has a state that can change with time according to rules. We start by considering random walk simulations and having projects on the formation of polymers and crystals. Additional important CA algorithms involve diffusion and spreading, and simulation of the formation of biofilm structures provides a significant application of these techniques. Moreover, at the end of the term, students

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have a brief introduction to concurrent processing and parallel algorithms. Consideration of more complex and larger biofilm arrangements illustrate the importance of high performance computing to computational science. In this paper, we discuss instruction using the curriculum module "Biofilms: United They Stand, Divided They Colonize," which the authors developed for the National Computational Science Institute (NCSI) Undergraduate Petascale Education Program (UPEP) [4].

2. Scientific Question

Stones in streams, teeth, water and sewer pipes, and the breathing passages of cystic fibrosis patients are all covered or lined with biofilms, assemblages that prove absolutely critical to life. Biofilms are communities of very small organisms that adhere to a surface (substratum) in an aqueous environment [5]. These organisms are usually bacteria, but algae or fungi may also form biofilms. Sometimes, these groups may even be mixed. The organisms are not only attached to a substratum, but are linked with each other within a matrix of biopolymers (polysaccharides, proteins, lipids, and nucleic acids). It is now believed that the vast majority of microbes are not solitary, planktonic, as was once assumed. Most microbial life seems to be part of one of these communities, and the planktonic forms may be simply ways to colonize other surfaces. We also know now that the free-floating members of each species are phenotypically quite different from their socially connected counterparts [6].

Dental plaque is a fitting example of a biofilm [7]. Forming on the surfaces of the teeth and soft tissues of the oral cavity, it is linked to dental caries (cavities), periodontal diseases, and even cardiovascular disease [8]. Examination of plaque reveals a complex architecture with a heterogeneous array and dispersal of cells within a matrix associated with fluid-filled spaces.

Biofilms are ubiquitous, and dental plaque is only one example. Given their prevalence, there must be some advantages for microbes to band together into such communities. In fact, there is quite a list of advantages. For a human pathogen like *Pseudomonas aeruginosa*, a common cause of respiratory diseases, living in biofilms greatly increases the success of infection [6]. Biofilms afford greatly increased protection from antibiotics, the host's immune system, and physical injury. According to Costerton, 65-80% of all bacterial diseases in human beings are from chronic biofilm infections [9]. For years, most physicians and scientists conceived of bacterial diseases derived from the single-celled, planktonic form of the microbe. Medical treatment was gauged to combat such forms, not biofilms. So, it is little wonder that we are finding increasing difficulties in combating pathogens.

For the most part, biofilms seem to be a threat to our species, although we are beginning to utilize them in positive ways for bioremediation and wastewater treatment for "green" buildings. Because these communities have such important impacts on our lives, it behooves us to better understand the structure and function of biofilms [10]. Thus, developing a simulation of biofilm formation provides good motivation for students in Modeling and Simulation.

In the course, we simulated in two dimensions (2D) the formation of the structure of a biofilm without regard to its function. Each discrete time step of the simulation contains the following phases:

- Diffusion of nutrients
- Growth and death of microbes
- Consumption of nutrients by microbes

Consideration of these concepts provides the background for other projects, such as diffusion and release of microbial products, attachment to the biofilm of a microbe that is wandering in free space, and detachment of microbes from the biofilm, as well as applications in quite different areas.

3. Diffusion Model and Algorithm

Solution of the problem provides a meaningful application for a cellular automaton simulation, where a rectangular grid of cells represents the world and rules (or transition rules), specifying local relationships and indicating how cells are to change state, regulate the behavior of the system. An advantage of such grid-based models is that we can visualize through informative animations the progress of events.

3.1. Diffusion Model

In modeling the nutrients in the system, we assume that we have a homogeneous collection of nutrients that is completely mixed at a constant temperature in which the nutrients diffuse at the same rate throughout the system.

As with many simulations, we model the dynamic area under consideration with an *m*-by-*n* grid (or $m \times n$ grid), or lattice or a two-dimensional array, of numbers. Each cell in the lattice contains a value representing a characteristic of a corresponding location.

For the diffusion model, the state of a cell depends on the states of its eight nearest neighbors. We can say that the change in an cell's nutrient value, $\Delta site$, from time t to time $t + \Delta t$ is a **diffusion rate parameter** (r) times the sum of each difference in the nutrient value of a neighbor and the cell's nutrient value (*site*), which simplifies to the following expression, where $0 < r < 0.125$:

$$(1 - 8r)site + r(\text{sum of nutrient value of eight neighbors}) \quad (1)$$

3.2. Boundary Conditions

The biofilms model provides ample motivation for the study of boundary conditions because the simulation employs a combination of these conditions. Suppose that the surface to which the biofilm adheres, or substratum, is on the left and an infinite supply of nutrients occurs on the right. For this infinite supply, the expanded nutrient grid has an east-most (right) column with each cell having constant nutrient value. In this same grid with no nutrients present on the surface, we have a west-most (left) column of all zeros. We use periodic boundary conditions in the north and south directions so that part of the nutrient in the north diffuses to the south and vice versa.

3.3. Diffusion Algorithm

Diffusion occurs on the nutrient grid at each time step. We initialize this grid to be an *m*-by-*n* matrix with each element having a dimensionless constant value, *MAXNUTRIENT*. For ease of visualization, we have $0 < MAXNUTRIENT \leq 1$. A function, *diffusion*, takes the diffusion rate and the nutrient values of a cell and its eight neighbors and returns the result of Equation (1).

For calculation of new values along the edges, we must extend the boundaries of a grid. As indicated earlier, we have periodic boundary conditions in the north-south directions, constant 0 in the west direction containing the substratum, and constant *MAXNUTRIENT* in the east direction with its endless nutrient supply. A function takes an *m*-by-*n* matrix and returns such an extended $(m + 2)$-by-$(n + 2)$ matrix.

After extending the grid by one cell in each direction using these boundary conditions, we apply the function *diffusion* to each internal cell and then discard the boundary cells. To do so, we define a function that takes an extended square lattice and returns the internal lattice with *diffusion* applied to each site.

We can simulate diffusion nutrients by initializing a grid with of values and repeatedly extending the grid, applying *diffusion* to each internal cell, and returning a new grid. An animation of visualizations of the grids illustrates the results.

4. Projects in Other Areas

With only a discussion of diffusion and an earlier consideration of random walk simulations, students are prepared to cover a variety of other applications. Thus, after covering the modeling and implementation of diffusion in the Modeling and Simulation course, teams of two or more students develop related projects, such as models for the applications in the next two sections.

4.1. Projects Simulating Mushroom Fairy Rings

Sometimes in a forest or yard, mushrooms seem magically to grow in circles, which we call "fairy rings." The mushroom is just the fruiting body of certain types of fungi. A fairy ring grows out from spores or transported soil

that contains fragments of mycelium, which forms the body of the fungus. The circular mass extends from this "nucleus". Circular or radial growth of the mycelium allows the fungus to move into unexploited areas in its search for nutrients. When the innermost part of the mass has exhausted the resources of that area, that portion becomes expendable. The outer mycelium removes and reabsorbs useful nutrients from the central region and transfers them outward. The innermost hyphae die and decay, thus forming a ring-shaped growth pattern. Simulations should illustrate the developing fairy ring including the various stages of the growing organism.

4.2. Projects Simulating Foraging

Almost all animals must navigate over short distances for such purposes as foraging. Evidence suggests that these animals might navigate to such a site using spatial relationships among nearby objects (landmarks). This series of markers form geocentric references that are stored in memory as a neural representation, or **cognitive map**, of the environment.

For these projects, a grid represents the cognitive map, and each cell contains whether the cell contains a barrier, an "animal", or neither and an expectation value. If a cell contains an animal and a reward event occurs, such as finding food, then the expectation value becomes some reward value, say 1. In contrast, if a non-reward event, such as not finding food, occurs in an animal cell, then the expectation value becomes 0.

For the first phase of the simulation, each cell of a grid (cognitive map) is initialized to some very low, uniform expectation value. The animal searches with a random walk for the food or nest until found. The second phase allows diffusion to continue without the animal. Third, the animal starts in the same location as in the first phase, and the simulation runs as continually the animal moves to the neighboring cell with the highest expectation value and diffusion occurs.

Experiments have shown that the animal goes directly to the initial location of the food. Not finding the stash, the animal begins searching around the area where the food was. Its path appears erratic and looping and gradually moves further from the former stash site. Simulation results should mimic this behavior.

5. Model and Algorithm for Biofilm Growth

The remainder of biofilm simulation involving growth and consumption of nutrients can be covered before, after, or while other projects, such as those in Section 4, are developed by the students.

5.1. Biofilm Growth Model

For modeling the bacteria in biofilms, we employ an identically shaped grid to that of the nutrient grid, and cells in the same position in the two grids represent the same location. For example, the cell in row 3 and column 7 of the bacteria grid indicates the bacterial state (empty, bacterium, dead bacterium), while the corresponding cell in the expanded nutrition grid indicates the amount of nutrients there.

A cell of the bacteria grid can be in one of three states: contain a live bacterium, contain a dead bacterium, or be empty. As with the nutrient grid, we have periodic boundary conditions in the north-south direction. In an extended bacteria grid, the far west (left) direction has an edge of border cells indicating the substratum, and the far east (right) direction also has an edge of border cells that do not accommodate growth from the interior.

If a location with a bacterium has no nutrition, the bacterium dies of starvation. Cells with dead bacteria remain in that state from one time step to the next. With a certain probability, usually related to the amount of available nutrition, a live bacterium divides at random into a neighboring empty cell.

5.2. Growth Algorithm

To represent the four states of a cell in the extended bacteria grid, we employ the following values in the constants that are in parentheses: 0 (*EMPTY*) to indicate an empty cell with no bacterium, 1 (*BACTERIUM*) for a cell with a bacterium, 2 (*DEAD*) to represent a cell with a dead bacterium, and 3 (*BORDER*) for a border cell.

For the simulation studied by the class, the initial bacteria grid is an *m*-by-*n* matrix with bacteria (value *BACTERIUM*) occurring at random in the first column and all other cells being empty (value *EMPTY*).

For growth of a bacterium, we use periodic boundary conditions in the north-south directions, a first column with each cell having the value *BORDER* indicating a surface to the west, and a last column with each cell having the value *BORDER* so that bacteria do not grow to the east. A function takes an *m*-by-*n* matrix parameter and returns an $(m + 2)$-by-$(n + 2)$ matrix with appropriate boundary conditions.

For some constant, $0 < p \leq 1$, our simulation calculates the probability that a cell divides as p((cell's nutrition)/(sum of nutrition values in all cells with live bacteria)).

6. Model and Algorithm for Consumption of Nutrients

At each time step, a bacterium consumes a constant amount of nutrient. For this simulation, nutrition is only consumed in the cells containing bacteria. In each such cell, a bacterium eats a constant amount (*CONSUMED*) of nutrients, so that the new value for the cell's nutrient is the old value minus *CONSUMED*. However, a bacterium cannot consume more than is there; so that if the difference is negative, we use 0.0 instead.

7. Simulation

To perform the simulation of a biofilm's structural formation, we define a function *biofilm* with parameters *m* and *n*, the number of grid rows and columns, respectively; the probability of a bacterium in an initial bacteria grid's first column element; the rate of diffusion of nutrients in the nutrient grid; *p*, the constant ($0 < p \leq 1$) used in the calculation of the probability that a bacterium divides; and *t*, the number of time steps. The function *biofilm* returns two lists, a list of the initial bacteria grid and the next *t* bacteria grids in the simulation and a corresponding list of nutrient grids.

At the beginning of the simulation, the bacteria and nutrition grids are initialized and stored in lists, *bacGrids* and *nutGrids*, respectively. For each time step, the nutrient grid is extended using appropriate boundary conditions and the diffusion function is applied to each internal cell. Then, bacteria can grow or die, resulting in a new bacteria grid. Afterwards, the bacteria consume nutrients, so that we have a new nutrition grid. Each of these grids is stored in the appropriate list, *bacGrids* or *nutGrids*.

8. Display Simulation

Visualization helps us understand the meaning of the grids. For each bacteria grid in the first list returned by *biofilm*, we generate a graphic for a rectangular grid with colors, such as yellow representing an empty site; green, a bacterium; and dark gray, a dead bacterium. A function with a parameter of the list of lattices from the simulation produces these figures. We animate the sequence of graphics to view the changing biofilm scene.

Because nutrient values are on a continuum from 0 to 1, we employ a grayscale for the animation of nutrient diffusion. On such a scale, 0 is black and 1 is white. So that the higher nutrient values appear darker, we subtract each nutrient value from 1 to obtain its degree of gray.

9. Rubric for Assessment

Figure 1 displays bacteria and nutrient grids at a later time step in a biofilm simulation. For the bacteria grid on the left, live bacteria are in dark gray, while dead bacteria are black. In the nutrient grid on the right, cells with a larger amount of nutrient are blacker. The latter grid illustrates the bacteria's gradual consumption of food as well as the diffusion of nutrients.

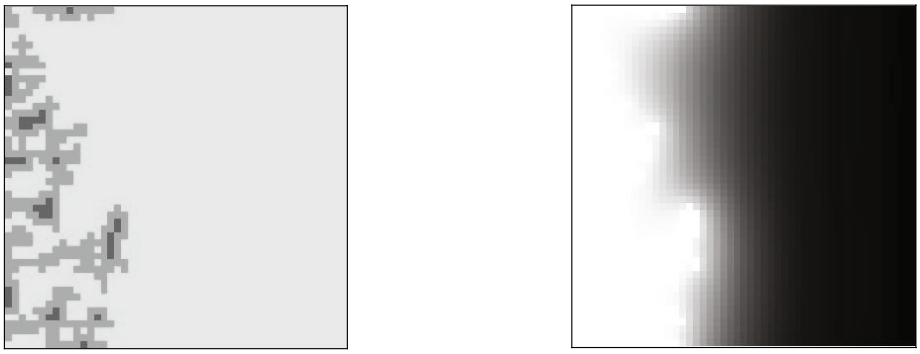

Fig. 1. (a) a bacteria grid; (b) corresponding nutrient grid

As the International Water Association points out, "Most biofilm models today capture only a small fraction of the total complexity of a biofilm system, but they are highly useful" [11]. We chose in the *biofilm* simulation only to model structural formation, not function. Simulation results agree with various features of biological biofilms. For example, as Figure 1 shows, with time, the overall thickness increases, and inert (dead) areas are greater near the substratum [12].

As Christoph Schaudinn et al. so eloquently states, "magnified views reveal microcolonies in an English garden to topiary delights, taking shapes that resemble mushrooms, towers, and arboreal structures…" [13]. Researchers have also observed other interesting features, such as pores, in biofilms [14]. Figure 1 displays these phenomena. The nutrient grid and simulation rules based on reality provide explanations for some of these shapes. Bacteria have consumed much of the nutrients towards the substratum in the west; nutrients continually come from the east; and bacteria divide at higher rates in nutrient-rich environments.

However, allowing our simulation to run for many time steps can reveal some anomalies not generally present, such as very long dendritic structures. Refining the model to account for erosion of surface bacteria should ameliorate this situation.

Also, the current model does not show water channels present in so many biofilms and does not indicate the biofilm's density, which is significantly greater near the substratum. Moreover, in our simplifying assumptions, we ignored important aspects, such as hydrodynamics, extracellular polymeric substances (EPS), chemical oxygen demand, and heterogeneity. Accounting for such features can enlighten our understanding of biofilms but can result in significantly more complex models that require much greater computing resources. Other types of models, such as continuum or discrete particle-based models, are advantageous for showing such features and for taking into account biofilm function.

10. Computing Power

Biofilms are highly complex with numerous features. We have chosen to consider form, not function, in 2D and have employed a number of simplifying assumptions. The simulations were run on a personal computer. However, with larger grids, conversion to 3D, and refinement to more complex models, simulations can stretch computing resources significantly. For example, Chrysi S. Laspidou and Bruce E. Rittmann in referring to biofilm models

involving hydrodynamics states, "Although such 2d models are now accessible for ordinary personal computers of nowadays (even for time-dependent problems a few minutes may be sufficient), the 3d problems of such type are at limit and better require parallel computing power" [12]. Thus, it is advantageous for the modeler to be able to use high performance computing (HPC) when needed.

11. Conclusion

The structural growth of biofilms provides a meaningful application for computational science undergraduate or graduate students. Understanding of concepts, such as boundary conditions, diffusion, and the iterative process of simulations, enable students to develop projects in a number of other application areas. Moreover, extensions of the illustrated biofilm simulation motivate the need for implementations using high performance computing in computational science.

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